(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number : **0 475 760 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number : **91308339.0**

(51) Int. Cl.[5] : **G01N 27/447**

(22) Date of filing : **12.09.91**

(30) Priority : **13.09.90 US 581683**

(43) Date of publication of application :
18.03.92 Bulletin 92/12

(84) Designated Contracting States :
CH DE FR GB IT LI SE

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(54) Method and device for high speed separation of complex molecules.

(57) A high speed electrophoresis apparatus having various features is disclosed. A capillary tube 100 has a region 402 of reduced diameter so that the voltage drop (V/cm) along the reduced diameter portion is greater than for other parts of the tube, thus reducing the total voltage drop which must be applied along the tube. A sample is tabbed with a fluorescent material and is irradiated by a laser beam 105 to photodegrade the tag, providing on-column optical gating of the sample, a probe beam 106 detecting the non-irradiated sample. The sample introduction may be modulated by control of the laser or conventional microswitching using a computer and the modulation waveform compared to the detection output, allowing for multiplex operation.

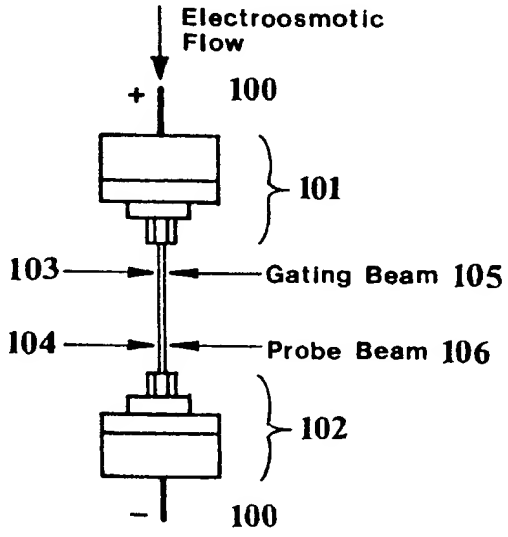

figure_1.

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This invention relates generally to the fields of electrophoresis and chromatography. More particularly, it concerns an improved process and apparatus for sample introduction and high-speed separation of complex molecules.

Electrophoresis has been widely used for several decades as a method for separating ionized compounds. More recently, there has been growing interest in capillary electrophoresis (CE) as a general, high-efficiency means of separating complex mixtures. In CE, the separation is carried out in a capillary tube with a typical inner diameter of 5 to 100 μm and a total length of 30 to 100 cm. The small radial dimensions of the capillary allow Joule heat to be dissipated efficiently, which in turn allows potentials as high as 30 kV to be applied across the length of the capillary. As a result, excellent separation efficiencies (> 1,000,000 theoretical plates) have been reported for many compounds, often in analysis times as short as a few minutes. When compared with chromatographic separation procedures, CE can offer a significant improvement in both speed and efficiency for the separation of charged species.

With a typical CE instrument, separation of a mixture usually requires between 5 and 30 minutes. Although this time is fast relative to many competitive procedures, it is slow relative to many chemical events. Another problem is that the traditional methods of sample introduction (e.g., electromigration and hydro-static pressure) are relatively slow and unwieldy. As a result, CE has not been used as a method for monitoring dynamic chemical systems. To gain this capability, it is necessary to increase the speed of the analysis. One method for achieving this goal is to reduce the time required for the electrophoretic separation. This cannot be achieved by simply increasing the potential across the capillary (and maintaining the same capillary length) or by decreasing the length of the capillary (and maintaining the same potential). Increasing the potential leads to excessive Joule heating and decreasing the length of the capillary without increasing the field strength adversely affects resolution.

It is an object of the present invention to provide an improved device and method for increasing the speed of electrophoretic and chromatographic separations. It is another object to provide an improved capillary system wherein an electric field can be concentrated in a particular section of a capillary column. It is a further object to provide a high-frequency sample modulation device that can be employed for multiplex chromatographic and electrophoretic separations.

The present invention provides an improved high-speed device and method that dramatically increases the speed of analysis of complex molecules with little loss of separation efficiency. In one aspect of the invention, capillaries of different diameters are used to concentrate an electrical field in a particular section of the separation column. This "enhanced" electrical field produces an increase in the electro-osmotic flow and electromigration of charged species which significantly reduces the time for analysis. Another aspect of the invention provides an on-column optical sample injection device for high-frequency modulation of sample introduction into a capillary column. This device can also be used for chromatographic analysis as well. Yet another aspect of the invention employs means for applying a substantially continuous stream of analyte to a separation column and means for modulating the analyte supplied so as to render the analyte detectable.

The rapidity with which an analysis can be performed makes the invention particularly well suited for multiplex separations. It is expected that because of the small size of the inventive instrumentation, an entire column, for instance, can be readily placed on a single substrate wafer (chip). This would facilitate column handling and permit integrated cooling and heating.

Aspects and preferred features of the invention are set forth in the claims and incorporated herein by reference.

Figure 1 is a diagram of a capillary mount showing the relative position of the capillary, the "gating" and the "probe" laser beams;

Figure 2 is a diagram showing the temporal relationship between the intensity of the "gating" beam and the fluorescence signal generated at the "probe" laser beam;

Figure 3 is a block diagram of a "fast" capillary electrophoresis instrument;

Figure 4 is a diagram demonstrating the principle of coupling capillaries of different diameters;

Figure 5 is a plot showing the effect of gating beam power on the fraction of fluorophore photolyzed;

Figure 6 is a plot of the current passing through the coupled capillary as a function of the electric field in the small diameter capillary. The buffer solution was 30 mM carbonate at pH 9.2;

Figure 7 is the graph of standard deviation of the fluorescein peak as a function of the electric field in a small diameter capillary. Column length (1) was 1.2 cm and the buffer was the same as listed in Figure 6. The electric field in the small diameter capillary was maintained at 2.7 kV cm^{-1};

Figure 8 is the graph of standard deviation of the FITC labeled arginine peak as a function of the sample introduction time. The buffer was the same as listed in Figure 6;

Figure 9 is an electropherogram of a mixture of FITC labeled amino acids. Sample introduction time was 40 ms. The buffer was the same as listed in Figure 6. Analyte concentrations are 10 μM for each species;

Figure 10 is an electropherogram of a mixture of FITC labeled amino acids. Sample introduction time was 50 ms. Analyte concentrations are 8 μM for each species. The buffer solution was the same as listed in Figure 6; and

Figure 11 shows a block diagram of a multiplex capillary electrophoresis apparatus.

Theory

Jorgenson and Lukacs [*Anal Chem.*, 53, 1981, 1298.] previously developed a set of equations which describe the efficiency and speed of capillary electrophoretic separations. These equations are:

$$N = \frac{\mu V_1}{2D} \quad (1)$$

$$t = \frac{L1}{\mu V} \quad (2)$$

where μ is the effective electrophoretic mobility of the analyte, V is the total applied voltage, V_1 is the voltage drop between the point of sample introduction and detection, L is the total length of the capillary in which the separation is being performed, 1 is the length of the capillary between the point of injection and the point of detection, N is the number of theoretical plates, D is the diffusion constant for the species, and t is the time required for the analyte to migrate to the detector. When discussing high-speed separations, it is desirable to develop a new equation which expresses the number of theoretical plates (N) which can be obtained in a unit period of time (t). This new equation can be derived from the two previous formulas:

$$\frac{N}{t} = \frac{\mu^2 V_1 V}{2D1L} \quad (3)$$

This expression can be simplified to:

$$\frac{N}{t} = \frac{\mu^2}{2D}\left(\frac{V}{L}\right)^2 \quad (4)$$

Thus, to maximize the number of theoretical plates obtained in any given time, the voltage to length ratio should be maintained at as high a level as possible. Nickerson and Jorgenson [*HRC&CC, 11*, 1988, 533.] demonstrated the utility of elevated V/L ratios by separating 8 amino acids in less that 70 seconds. In practice, what ultimately determines the V/L ratio is Joule heating of the capillary. Overheating of the capillary is recognized to produce broadened peaks [Lukacs, dissertation, University of North Carolina at Chapel Hill, 1983]. As this mechanism was not considered in the derivation of equation 4, the relationship between the indicated parameters are likely to change. To develop an expression for power dissipation in the capillary, first the current passing through the capillary (i) and the effective resistance of the buffer-filled capillary (R) must be calculated. Equations 5 and 6 allow these values to be defined in terms of fundamental parameters:

$$i = \frac{V\pi r^2}{\rho L} \quad (5)$$

$$R = \frac{\rho L}{\pi r^2} \quad (6)$$

where i is the current passing through the capillary, ρ is specific resistance of the buffer filling the capillary, and r is the radius of the central channel. By substitution, the power dissipation in the capillary can be calculated as shown in equation 7.

$$P = i^2 R = \frac{V^2 \pi r^2}{\rho L} \quad (7)$$

A more useful parameter is the power dissipated per unit length of capillary. Dividing through equation 6 by the column length (L) gives the following expression.

$$\frac{P}{L} = \left(\frac{V}{L}\right)^2 \frac{\pi r^2}{\rho} \quad (8)$$

Interestingly, the ratios N/t and P/L are both proportional to $(V/L)^2$. Thus, for all other factors remaining constant, N/t is proportional to P/L.

As a general rule, for a passively (radiatively) cooled column, a power dissipation of less than 1 Watt per meter of capillary produces negligible broadening of sample zones. It appears that the key to increasing the speed of a CE separation is to establish a set of experimental conditions in which the electric field is maintained at as high a level as possible, but where thermally induced zone dispersion is inconsequential. Equation 7 indicates that the power dissipation may be kept within acceptable limits simply by reducing the radius of the column. Although this procedure is very effective at minimizing power dissipation in the column, it can increase the difficulty of finding a suitable detector to record the passage of the analyte zone. An alternative procedure is to actively thermostat the column to help dissipate the excess heat [Nelson et al., *J. Chrom., 408*, 1989, 111]. For temperature stabilized capillaries, operation at power levels in excess of the previously stated limits may prove both practical and desirable.

INSTRUMENTATION

Sample Introduction by On-Column Optical Gating

Typically, in most capillary-based separations instruments, the sample is introduced as a "plug" of material at one end of the column and allowed to traverse the column where the separation occurs. With on-column optical gating, the components in the mixture to be determined are first tagged with a fluorescent molecule and then continuously introduced into one end of the column. As shown in Figure 1, capillary 100 is held in position by capillary supports 101 and 102. The polyimide coating on the portion of the capillary 100 situated between the supports has been removed. Near the entrance of capillary 100, at position 103, a laser (not shown) is used to photodeg-

rade the tag and thus render the material undetectable to a fluorescence detector which is located at position 104 further along the column. Position 103 is referred to as the point of sample gating where gating beam 105 is focused, and position 104, the point of detection, is where probe beam 106 is directed. As will be discussed further below, an advantage of the inventive device is that the distance between the point of sample gating and point of detection can be very short. This distance generally is less than 10 cm, and preferably between 1-5 cm. A sample zone is generated by momentarily preventing the laser from striking the column, and thereby allowing a small amount of tagged material to pass intact. Because the sample modulation is optical rather than mechanical, temporally narrow plugs of material can be formed in the column. Furthermore, the formation occurs while the capillary is maintained at the operating voltage. Separation of the tagged species occurs primarily in the column region between the point of sample gating and the point of detection. Because separation occurs throughout the length of the column between the electrodes, the level of separation of analyte in the narrow plugs formed can be varied depending on where along the column the gating point is chosen. Electrodes from the high potential source are situated in large buffer reservoirs at the ends of each column. Thus, a fluorescence signal will be recorded at the detector channel at some time delay from the interruption of the gating beam. This temporal relationship is illustrated in Figure 2.

On-column optical gating applies equally well to chromatography, especially, for instance, in high speed liquid chromatography separations of complex organic molecules.

Figure 3 shows a block diagram of the instrument used for high speed electrophoretic separations. An argon-ion laser 301 (Model 70-2, Coherent Inc.) operating at less than one watt of power at 488.0 nm is focused into the central channel of capillary 302 with a fused silica lens 307 (f.l. = 75 mm, Oriel Corporation) to photodegrade the fluorescent species. Intensity modulation of the laser beam is accomplished with an acoustooptic modulator 303 (Model AOM-30 Modulator and Model DE-30X VCO Driver, IntraAction Corporation). Hirschfeld [Applied Optics, 15, 1976, 3135] demonstrated that the molecule fluorescein could be efficiently photolyzed with relatively modest laser powers (~12 kW cm^{-2}). These power densities are easily achieved by focusing a low-power continuous wave laser into the capillary column. The experiments described herein employed molecules that are easily labeled with the fluorescein derivative, fluorescein isothiocyanate (FITC). The electrophoretic separation proceeds in much the same way as conventional CE with the effective column length (l) being the distance between the gating beam and the fluorescence detector.

Coupled Capillary Columns to Focus the Electric Field

It is normally difficult to work in high electric fields with short capillary columns. In particular, spontaneous breakdown in air may occur when the electric field strength exceeds 3000-4000 V cm^{-1}. As a result, special attention must be directed toward electrically isolating the buffer reservoirs. However, this problem is solved by coupling capillaries of different diameters to concentrate the electric field into a short section of small diameter capillary. This "coupled-column" technique is illustrated in Figure 4. In this embodiment, one end of capillary 402 (10 μm i.d., 4 cm in length) is inserted into capillary 401 (150 μm i.d., 85 cm in length). The other end of capillary 402 is inserted into capillary 403 (150 μm i.d., 14 cm in length). The capillaries were bonded at the two connections. The equivalent electrical circuit is shown to the left of the column diagram. Analysis of this circuit indicates that approximately 90% of the voltage drop occurs over the 4 cm length of capillary 402. Consequently, it is possible to generate electric fields in excess of 5000 V cm^{-1} in the short length of capillary 402. Typically, electric fields of only 300 to 400 V cm^{-1} are employed with CE. As discussed previously, this elevated electric field can be used to shorten the time of analysis. However, the overall length of the coupled capillary is the same as the length of typical capillaries used in the traditional CE analysis so problems with isolating the high voltages are minimized.

As discussed previously, Joule heating of the capillary must be minimized if separation efficiency is to be maintained. Capillary diameters between 5 and 15 μm seem to provide a good compromise between capillary temperature control and ease of use with a fluorescence detector.

Although the above embodiment employs capillaries of different diameters which are bonded together to achieve the electric field focusing effect, other geometries should be feasible. Specifically, it is possible to vary the diameter of the capillary during the drawing process or in etching a capillary of differing diameter in a non-conductive substrate (e.g., silicon wafer). The latter also makes column handling much easier and permits integrated cooling or heating. It is also possible to eliminate either capillary 401 or capillary 403 and employ a capillary means with two sections of different diameters. As long as the sample and electrolyte in a first section have a higher electrical resistance than that of the sample and electrolyte in the second section, the electric field strength in the first section will be higher than that in the second section to achieve a more rapid separation.

Apparatus for Detection and Signal Processing in High Speed CE Employing On-Column Optical Gating and Coupled Capillary Columns

As shown in Figure 3, a small fraction (~4%) of the laser power is split from the laser beam 304 and directed into the capillary 302 to form a fluorescence detector (the probe beam 106 in Figure 1). Specifically, a fraction of beam 304 is split by beam splitter 305 into gating beam 105 and probe beam 106. The gating beam is focused onto the capillary by lens 307. The probe beam is focused onto the capillary by mirror 306 and through lens 308. (As shown in Figure 1, the gating beam and probe beam are focused on different positions on the capillary.) The resulting fluorescence signal is collected with a microscope objective 309 (16x, Melles Griot) and then spectrally isolated with a monochromator (Model H-10, Instruments SA Inc.) and bandpass filter (Omega Optical), together shown as 310. The resulting photon flux is converted to an electrical signal with photomultiplier tube (Model R1527-03, Hamamatsu) and a high speed amplifier (Model 427, Keithley Instruments), together shown as 311. A LabVIEW (National Instruments) program running on the Macintosh II computer acquired the data through a laboratory interface board (Model NB-MIO-16XL-42, National Instruments) configured with a 16-bit analog to digital interface. This same program is used for data processing and storage. Peak parameters (theoretical plates, retention time, peak widths, etc.) were derived from statistical moments that were calculated with a second LabVIEW program.

Procedures for Preparing Fluorescently Tagged Molecules

Solutions were prepared in the following manner. First 1 mL of FITC/acetone solution (6.1 mM) is added to 3 mL of 3 mM solution of each amine in a pH 9.2 carbonate buffer. This mixture is allowed to react at room temperature for at least three hours. This mixture is further diluted with the mobile phase (pH 9.2, carbonate buffer) to obtain the desired concentration of labeled product. Before use, all solutions were passed through a 0.22 μl filter to remove particulates.

Sample is introduced into the capillary by electrophoretic migration. Although this sample introduction procedure necessarily selects for those species with the highest electrophoretic mobility, for all molecules studied it was found that the injected amount was adequate for the experiments. Before data collection was initiated, the sample in buffer was passed into the column for several minutes to equilibrate the capillary with the solution. Complex molecules which may be separated with the present invention include charged molecules such a proteins, glycoproteins, peptides, amino acids, and polynucleic acids.

Fluorescent molecules which may be used in connection with this invention are exemplified by fluorescein and fluorescein derivatives, dansyl chloride (5-dimethylaminoonaphthylene-1-sulfonyl chloride) and analogs thereof, coumarin and coumarin analogs, and fluorescamine. Numerous other photobleachable fluorescent molecules are available.

RESULTS FROM THE SEPARATION OF FITC LABELLED ARG, PHE, AND GLU

The success of on-column optical gating relies upon its ability to efficiently photodegrade the fluorescently-labeled compound as it passes through the gating beam. To assess the degree to which complete photobleaching was observed, a bare fused silica capillary (10 μm i.d.) was employed with the inventive apparatus described. FITC labeled arginine was forced through the capillary at a constant velocity (0.19 cm s^{-1}). The relationship between probe beam power and the fraction of the fluorophore photolyzed is illustrated in Figure 5. These data suggest that at least two processes are involved in the photolysis of fluorescein. The first mechanism is fast and irreversible. This accounts for the rapid drop in fluorescence intensity observed at low laser powers. The second mechanism is less sensitive to laser power and accounts for the elevated fluorescence levels observed even at high laser powers. Unfortunately, this persistent fluorescence introduces a background upon which all the signals must be observed. Not surprisingly, this background increased the noise which limits the dynamic range and precision of the measurements. However, there is no reason to believe that other fluorophores would suffer from these same limitations.

Figure 6 shows the measured current passing through the coupled capillary column as a function of the electric field in the short capillary. Significant deviations from linear behavior are observed by the time the field strength has reached 3 kV cm^{-1}. This behavior is indicative of Joule heating of the buffer. Figure 7 supports this hypothesis by plotting the temporal standard deviation of the sample zone as a function of the electric field. A minimum zone width is observed when the applied voltage is 2.0 kV cm^{-1}. This problem can be partially overcome by reducing the current passing through the capillary. This is easily accomplished by lowering the concentration of the supporting electrolyte in the buffer or by further reducing the diameter of the capillary.

In Figure 8 the temporal standard deviation of the peak for FITC labeled arginine is plotted as a function of the sample introduction time (the time the "gating" laser beam is deflected away from the capillary) for a capillary with a length (l) of 1.2 cm and an electric field of 3.3 kV cm^{-1}. These data demonstrate that

temporally small sample zones must be injected into the capillary to obtain a minimum peak width. As illustrated in Figure 8, the sample introduction time is preferably less than about 40 milliseconds.

Figure 9 shows the electropherogram acquired when a solution containing FITC derivatives of three amino acids (Arg, Phe, Glu) was introduced into the instrument. For this analysis, the distance between the "gating" and the "probe" beams was 1.2 cm. The electric field was maintained at 3.3 kV cm^{-1}. The elution time for these three species are 0.62, 0.91 and 1.33 seconds respectively. The theoretical plates for these peaks ranged between 5000 and 7000.

Separation of the amino acids (Arg, Phe, Glu) had been reported by Cheng and Dovichi [Science, 242, 1988, 562]. In that report, the elution time for the amino acids ranged between 13 and 24 minutes with peak efficiencies of approximately 400,000 theoretical plates. Thus, a decrease in separation efficiency accompanies the increase in overall speed of analysis. Heat buildup in the capillary likely has contributed to some of the width of the peak observed in Figure 9. The inventive capillary device can accomodate electric field strengths up to at least 5000 V/cm^{-1}. Moreover, with integrated (i.e., active) cooling significantly higher fields can be accomodated. In principle, reducing this excess heat should allow even higher separation efficiencies to be obtained.

To regain some of the efficiency sacrificed the operating conditions were modified. Figure 10 shows the electropherogram obtained when these same compounds were electromigrated in a field of 1750 V cm^{-1} over a distance of 4 cm. The efficiencies of the three peaks ranged from 70,000 to 90,000 theoretical plates which corresponds to approximately 2 x 106 plates per meter and a HETP of 0.5 μm. Arginine was found to be separated at a rate equivalent to 12,000 theoretical plates per second. Equally important, the peak capacity of the electropherogram has been dramatically increased.

Application of High Speed CE with Multiplex Separations

Multiplex separation techniques provide an alternative to the batch separation mode (single injection input signal) employed in the vast majority of separation procedures. Both techniques produce essentially identical information (i.e., data about individual components in a mixture). However, the multiplex mode of operation can provide some very important advantages when the analyte to be determined is near the detection threshold or when transient events must be monitored [Annino and Grushka, *J. Chromatogr. Sci.*, 14, 1976, 265; Phillips, Anal. Chem., 52, 1980, 468A].

In the multiplex separation procedure, multiple sample injections are made into the carrier stream. Ideally, each injection results in a separation of the mixture components just as in the single injection mode. However, peaks from these separations can be strongly overlapped at the detector and are therefore not directly interpretable by the analyst. However, if the injection function is known, the separation information can be recovered (e.g., by cross-correlation or deconvolution). The primary advantage of this detection mode is that sample throughput is enhanced which in principle will reduce the limits of detection for any given species. Furthermore, because a separation does not have to be complete before the next injection is made, monitoring of transient signals can be accomplished with relative ease.

Several problems have inhibited the development of multiplex separation procedures for chemical analysis. Most multiplex methods employ computers for experiment control and data interpretation. Only recently has the widespread availability of powerful personal computers overcome this problem. A more fundamental problem, particularly for chromatographic separations, is loading of the stationary phase by the multiple sample injections. The resulting non-linear partitioning of the solute between the stationary and the mobile, phase can make quantitative interpretation of the recorded signal difficult if not impossible. To its advantage, CE does not involve the partitioning of the solute between phases, and therefore, is not susceptible to this problem. The"high-speed" CE instrument described above is particularly well suited for this application because of the rapid analysis times and simplicity of making multiple injections onto the column. Although multiplex separations perform particularly well in electro-phoresis, multiplex chromatographic systems employing the inventive instrumentation should also yield good results. This is particularly the case with gas chromatography where equilibrium between the mobile and stationary phases is achieved relatively quickly.

Multiplex procedures provide a unique method whereby the limits of detection of time resolution of a separation can be enhanced. Figure 12 shows a block diagram of a multiplex capillary electrophoresis apparatus. In this embodiment, modulation device 1206 provides multiple sample injections of the analyte into capillary column 1200 where it is separated. Modulating device 1206 can comprise the above-described inventive on-column optical gating employing a laser or other conventional means such as microswitching. The effluent exits via waste stream 1209. Computer 1210 generates a pseudo-random modulation waveform which is used to control sample introduction into the capillary. A voltage power supply source 1207 maintains a high potential for separation. A detector 1208 is used to record the passage of the analyte peaks as they elute from the column. The detector output is digitized and stored by the computer. After data collection is complete, the mobility

spectrum is determined by cross-correlating the modulation waveform with the detector output. The cross-correlation is performed using the above-referenced LabVIEW program.

The multiplex technique described above employs the on-column optical gating device in an "on" and "off" mode, which can be represented as square waves. However, multiplex separation can also be accomplished if samples are introduced continuously and in varying concentrations, which can be represented, for example, as a sine wave, or a pseudo-random waveform. Where the passage of analyte peaks from different samples overlap, a pseudo-random modulation injection waveform (for example, pseudo-random pattern of turning the laser on and off) will enable the peaks to be identified using the LabVIEW Program Data Recovery techniques employing conventional pseudo-random waveforms.

It is to be understood that while the invention has been described above in conjunction with preferred specific embodiments, the description and examples are intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims.

Claims

1. An electrophoretic device for applying an electric field to perform electrophoretic separation of a sample, comprising:
   a capillary tube means having at least a first and a second section (401,402) having different cross-sectional dimensions, said capillary containing an electrolyte and the sample; and
   means for applying a voltage potential across the sample and the electrolyte in the two sections (401,402), wherein the dimensions of the first and second sections are such that the electrical resistance of the electrolyte and sample in the second section (402) is greater than that of the electrolyte and sample in the first section (401) to increase the electric field strength in the second section (402).

2. A device as claimed in claim 1, characterised in that the electrical resistance of the electrolyte and sample in the second section (402) is at least about an order of magnitude greater than that of the electrolyte and sample in the first section.

3. A device as claimed in claim 1 or 2, characterised in that the cross-sectional dimensions of the second section (402) are at least about an order of magnitude smaller than those of the first section (401).

4. A device as claimed in claim 1, 2 or 3, characterised in that the tube means is formed by connecting a first and second tube, where the inner diameter of the first tube (401) is approximately equal to the out diameter of the second tube (402).

5. A device as claimed in any one of claims 1 to 4, characterised in that the tube means further comprises a third section (403), wherein the electrical resistance of the electrolyte and sample in the second section (402) is greater than that in the third section (403).

6. A device as claimed in claim 5, characterised in that the cross-sectional dimensions of the first and third sections (401,403) are at least about an order of magnitude greater than those of the second section.

7. An on column optical gating device for introducing analyte into separating columns, comprising:
   a separation column (100) containing analyte including fluorescent molecules; and
   means (105) for photodegrading the fluorescent molecules to control the introduction of detectable molecules into the column (100).

8. A device as claimed in claim 7, characterised in that said photodegrading means (105) includes a larer (301).

9. A device as claimed in claim 7 or 8, characterised by an on-column detector (106) for detecting the fluorescent molecules.

10. A device as claimed in claim 9, characterised in that the photodegrading (105) means photodegrades the molecules at a location (103) in the column (100) and the detector (106) detects the molecules at a distance of less than about 5 cm from the location (103).

11. A device as claimed in claim 10, characterised in that the detector (106) detects the molecules at a distance of about 1 cm from the location (103).

12. A gating device for introducing analyte into a separation column (1200), comprising:
   means for applying a substantially continuous stream of analyte to said column; and
   means (1206) for modulating the analyte applied to the column (1200), so as to render the analyte detectable by a detecting process.

13. A device as claimed in claim 12, characterised in that said modulating means (1206) includes a micro-valve that modulates the amount of analyte supplied to the column (1200).

14. The device as claimed in claim 12, characterised in that said separation column (1200) contains analyte including fluorescent molecules, and said modulating means including means (105) for photodegrading the fluorescent molecules to control the introduction of detectable molecules into the column.

15. A high speed electrophoretic device comprising:
a capillary tube means (100) having at least a first and a second section (401,402) having different cross-sectional dimensions, said capillary (100) containing an electrolyte and the sample; and
means for applying a voltage potential across the sample and the electrolyte in the two sections, wherein the dimensions of the first and second sections are such that the electrical resistance of the electrolyte and sample in the second section (402) is greater than that of the electrolyte and sample in the first section (401) to increase the electric field strength in the second section;
means for introducing a detectable sample into the capillary tube means (100); and
a detector (106).

16. A device as claimed in claim 15, characterised in that said sample introducing means has a sample introduction time of less than about 40 milliseconds.

17. A device as claimed in claim 15 or 16, characterised in that said sample introducing means includes means (105) for photodegrading the fluorescent molecules to control the introduction of detectable molecules into the capillary tube means (100).

18. A device as claimed in claim 15, characterised in that the sample introducing means comprises an on column optical gating device (105) and the detector (106) measures fluorescence.

19. A device as claimed in claim 15, characterised in that said sample introducing means includes:
means for applying a substantially continuous stream of analyte to said column (100); and
means for modulating the analyte supplied to the column, so as to render the analyte detectable by a detecting process.

20. A device for multiplex separations, comprising:
a capillary (1200);
means (1206) for introducing a sample into the capillary according to a predetermined modulation waveform;
means (1207) for causing the sample to move through the capillary (1200) and for causing the sample to separate into its components;
means (1210) for modulating the frequency of sample introduction;
a detector (1210) for detecting the passage of sample components and for providing output signals indicative of such passage;
means (1210) for cross-correlating the detector output signals with said predetermined waveform.

21. A device for multiplex separations as claimed in claim 20, characterised in that the modulating means (1206) comprises on-column optical gating means (105).

22. A separation apparatus comprising:
a capillary column (1200);
input means for introducing a sample into the capillary column;
means (1207) for causing separation of the sample into its components;
detection means (1208) for detecting the separated sample components; and
modulation means (1206) for modulating the sample to limit the amount of sample components detectable by the detection means.

23. An apparatus as claimed in claim 22, characterised in that the modulation means (1206) comprises gating means (105) for selectively causing a portion of the sample to be substantially undetectable by the detection means upon separation.

24. An apparatus as claimed in claim 23, characterised in that the gating means (105) comprises means for intermittently focusing a laser beam at the sample in the capillary column.

25. An apparatus as claimed in claim 24, characterised in that the detection means (1208) comprises means (106) for detecting fluorescent tagged sample components, wherein the sample is tagged with a fluorescent material prior to separation and wherein the laser beam photodegrades a selected portion of the fluorescent tagged sample such that parts of separated sample components originating from the photodegraded portion of the sample are undetectable by the detection means (106).

26. An apparatus as claimed in claim 25, characterised in that the means (106) for detecting fluorescent tagged sample components comprises means for inducing fluorescence by influence of laser radiation.

27. An apparatus as claimed in any one of claims 22

to 26, characterised in that the means (1207) for causing separation comprises means for causing electrophoretic separation.

28. An apparatus as claimed in claim 27, characterised in that electrophoretic separation takes place along a section less than 10cm of the capillary column (1200) and the means (1207) for causing separation comprises means for applying an electric potential of greater than 1000 V/cm.

29. An apparatus as claimed in any one of claims 22 to 28, characterised by multiplexing means (1210) for controlling multiplex detection of separated components corresponding to multiple segments of the sample whereby the segment from which a particular sample component originates can be determined despite possible overlaps of the sample components.

30. An apparatus as claimed in claim 29, characterised in that the multiplexing means comprises means (303) for marking each segment with a different waveform and controlling the detection means to identify the waveform of each sample component thereby determining the originating segment of each sample component.

31. An apparatus as claimed in claim 30, characterised in that the means (313) for marking the segments comprises means (312) for controlling the modulation means to selectively, in accordance with the respective waveforms, cause part of each segment to be substantially undetectable by the detection means upon separation, thereby marking the segment.

32. An apparatus as claimed in claim 31, characterised in that the modulation means comprises means for intermittently focusing a laser beam at the sample in the capillary.

33. An apparatus as claimed in any one of claims 22 to 32, characterised in that the detection means (1208) comprises means (310) for detecting fluorescent tagged sample components, wherein the sample is tagged with a fluorescent material prior to separation and wherein a laser beam photodegrades a portion of the fluorescent tagged sample between adjacent segments such that parts of separated sample components originating from the photodegraded portion of the sample are undetectable by the detection means.

34. An apparatus as claimed in any one of claims 22 to 33 characterised in that the input means introduces a further sample in close sequence after the earlier sample has been introduced, the apparatus further comprising multiplexing means for controlling multiplex detection of the separated sample components whereby the sample from which a particular sample component originates can be determined despite possible overlaps of the sample components.

35. An apparatus for high speed separation of a sample into its components comprising:

a column having a capillary section (402) of reduced inside diameter; and

means for causing separation of a sample into its components comprising means for applying an electric potential along the column wherein separation takes place primarily in the capillary section (402) because of higher electric potential drop along the capillary section (402) than along the rest of the column.

36. An apparatus as in claim 35, characterised in that the column is an integral structure.

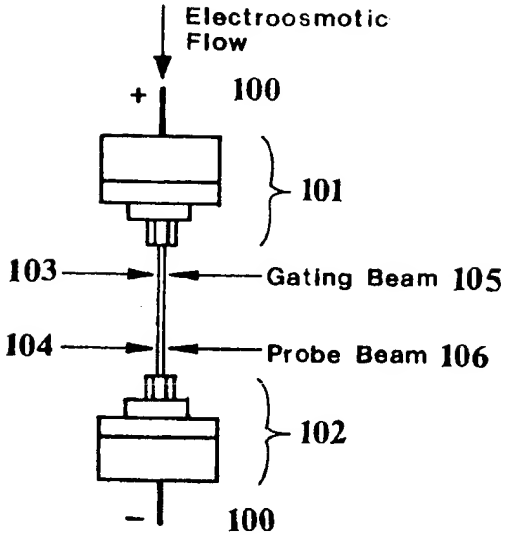
37. A method of gating an analyte in a separation column (100) comprising:

providing a substantially continuous stream of analyte in said column (100), said analyte including fluorescent molecules;

providing means (106) for detecting the fluorescent molecules in the stream;

photodegrading the fluorescent molecules in the stream upstream from said means for detecting the fluorescent molecules to thereby render the fluorescent molecules undetectable.

38. A method according to claim 37, characterised by the step of interrupting said photodegrading step to thereby render the fluorescent molecules detectable.



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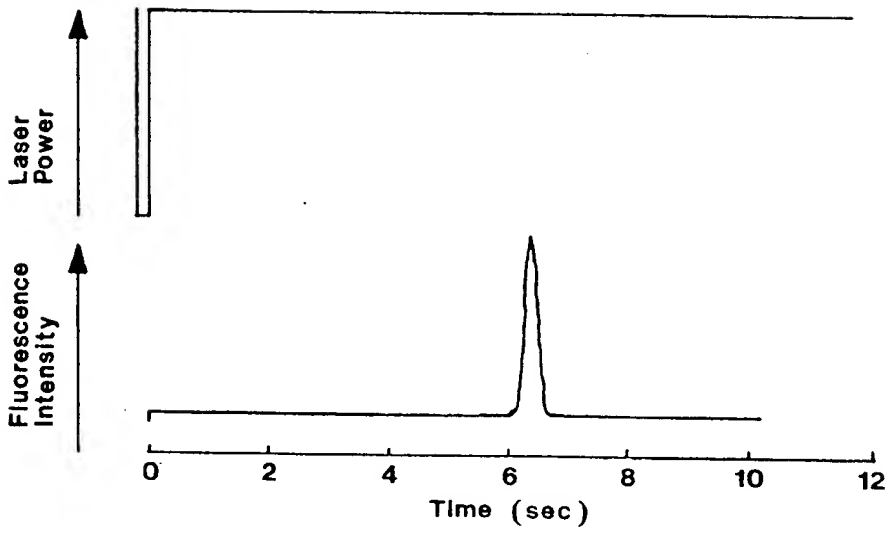
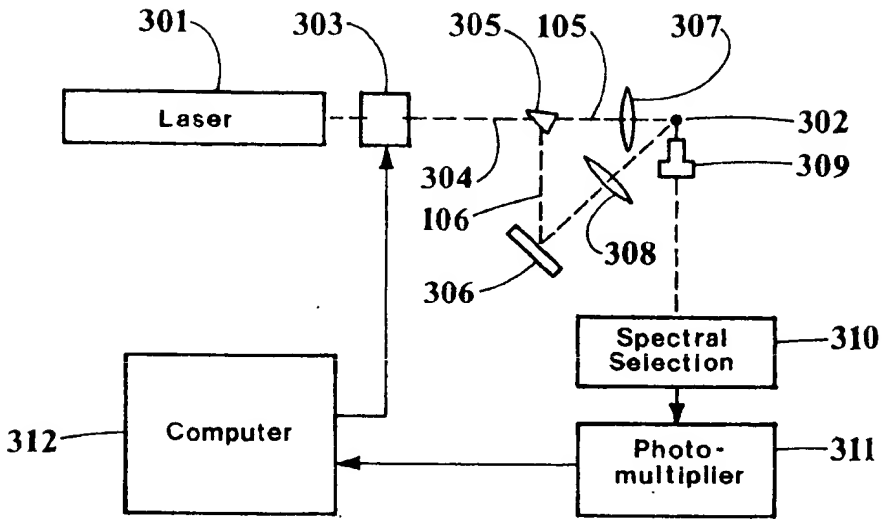


figure_2.



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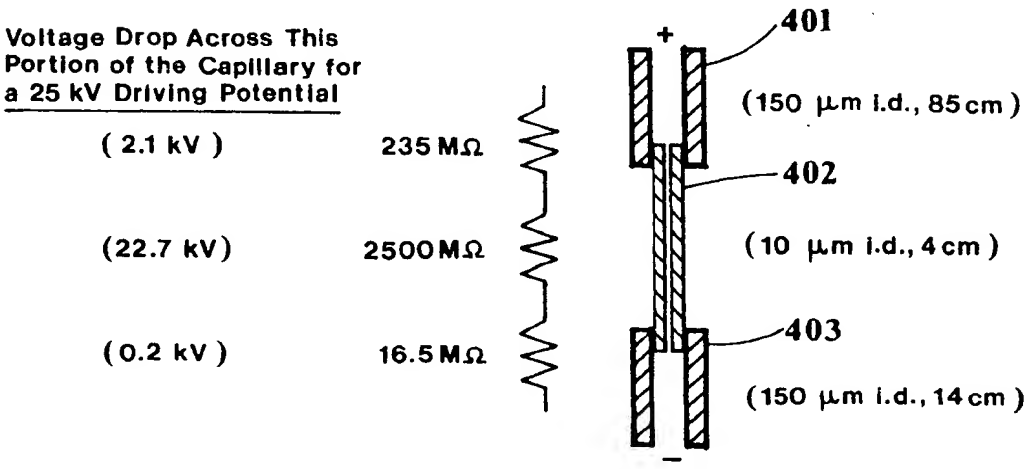

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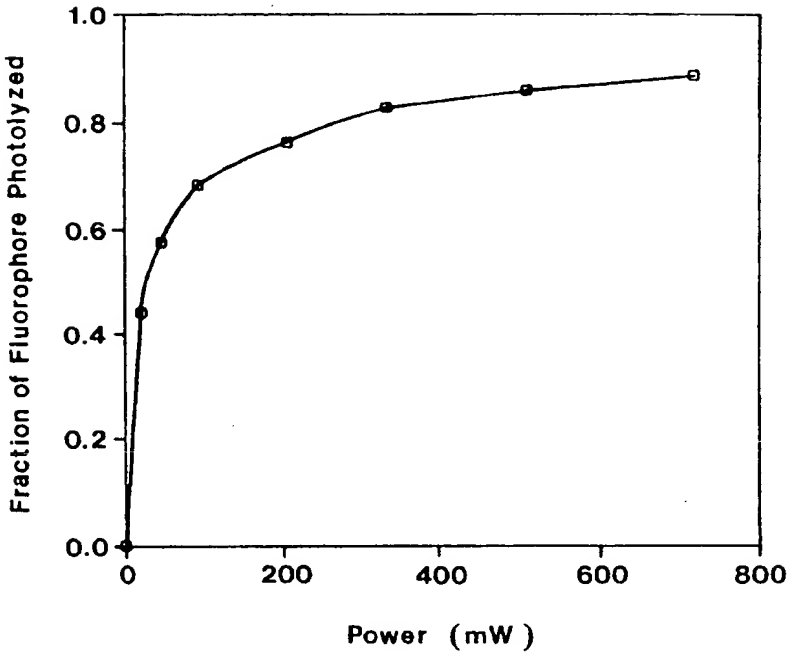

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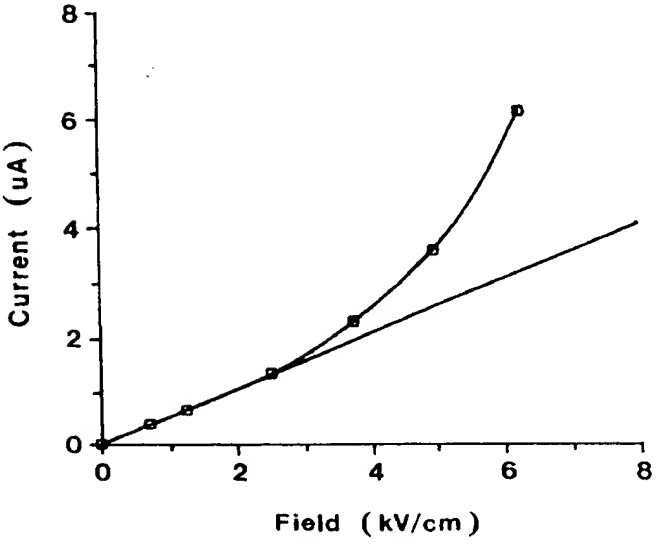

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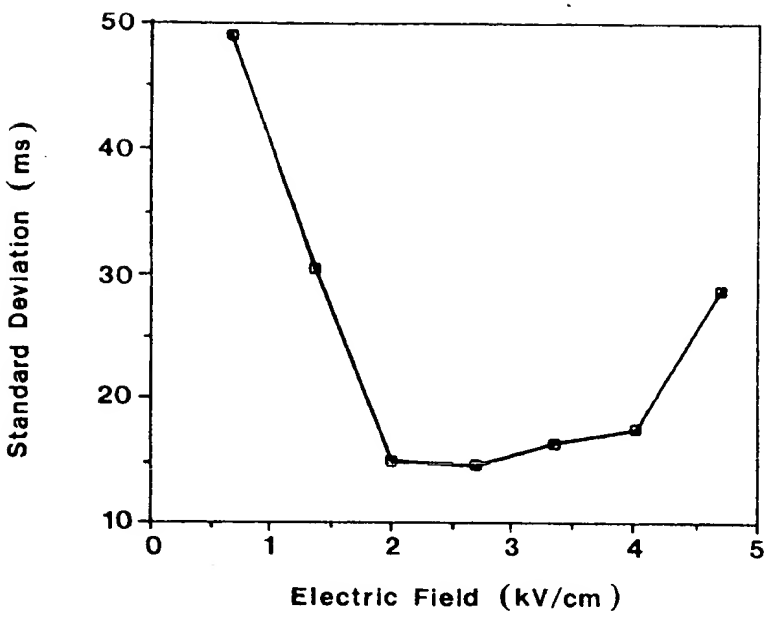


figure _ 7.

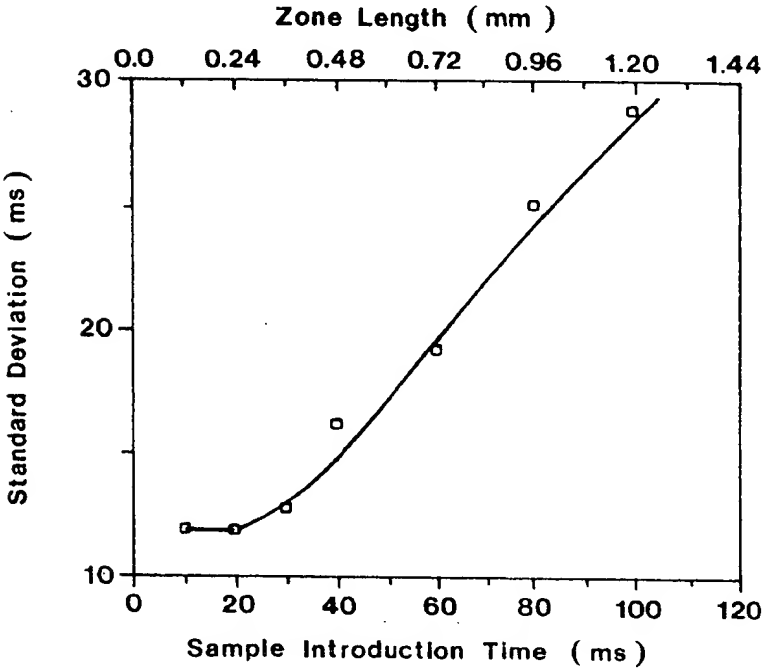


figure _ 8.

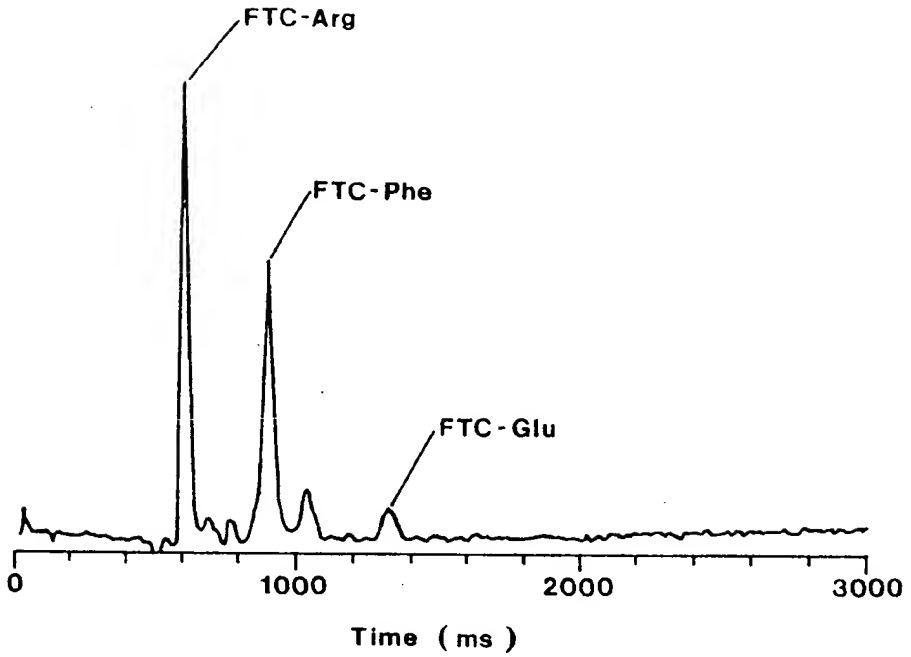
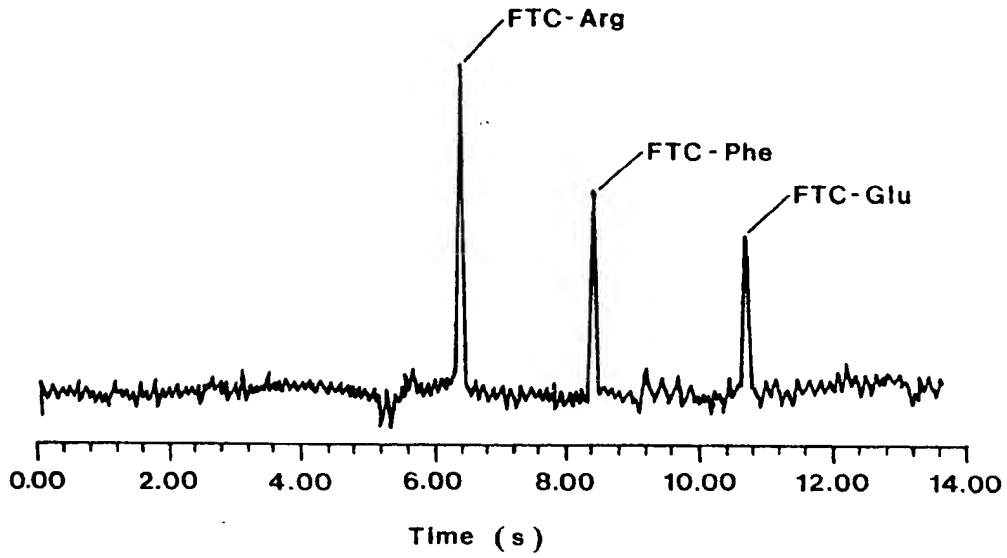


figure _ 9.



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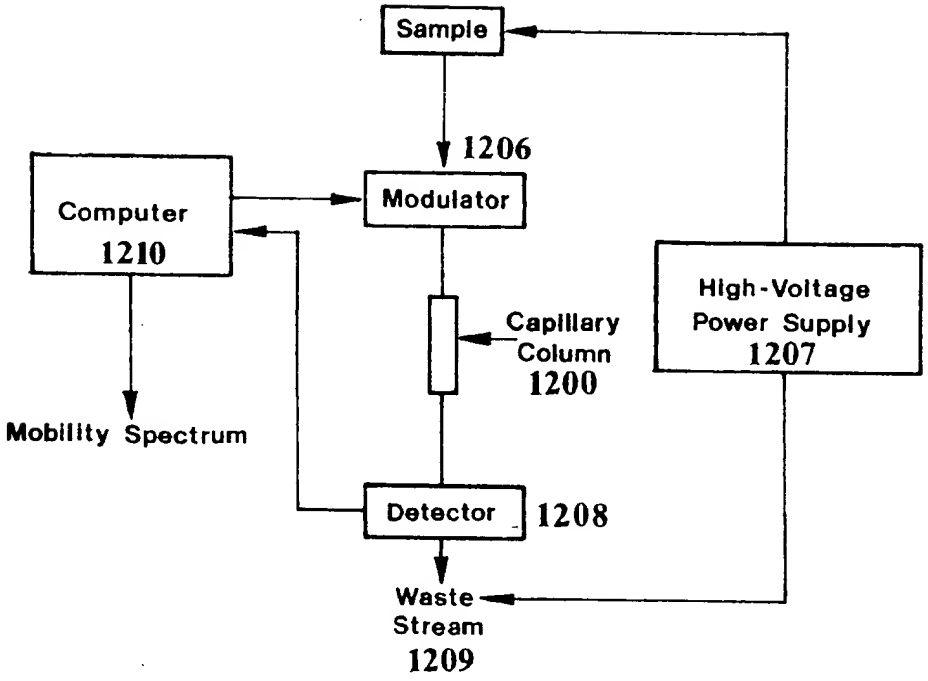
Sample
1206
Computer
1210
Modulator
High-Voltage
Power Supply
1207
Capillary
Column
1200
Mobility Spectrum
Detector
1208
Waste
Stream
1209

figure _ 11.